BIOMASS CONVERSION: FERMENTATION CHEMICALS AND FUELS*

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I. INTRODUCTION

Plant biomass is currently being researched as one of the desirable alternative raw materials to petroleum because it is readily renewable and abundant. The most abundant form of biomass on the planet earth is lignocellulose, which is composed of cellulose, hemicellulose, and lignin. Cellulose is our most abundant renewable resource and is available from sources such as wood, newsprint, urban waste, and manure. Of the 22×10^9 ton of cellulose generated by photosynthesis annually worldwide, about 4×10^9 ton per year (20%) is readily available for conversion to fuels, chemicals, or feedstuffs.[1] Cellulose derived from wood and grasses is the most available. Cellulose derived from sugarcane bagasse, corn refuse, and certain tree crops are produced economically; wood cellulose is currently the most economical.[2]

This article explores the current state of research on the conversion of cellulose, hemicellulose, and lignin by various processes to fermentable products, and the fermentation of these products to chemicals and fuels. A review of research activities is presented, including technologies available for the utilization of biomass, chemicals from fermentation processes, conversion of biomass to sugars, and direct bioconversion to liquid fuels.

II. LIGNOCELLULOSE — COMPOSITION AND DEGRADATION

Most plant fibers contain cellulose, hemicellulose, and lignin in approximate ratios of 4:3:3. Cellulose is a homogeneous polymer of glucose, whereas hemicellulose molecules are often polymers of pentoses (xylose and arabinose), hexoses (glucose and mannose), and a number of sugar acids.[3-6] Lignin, a polyphenolic macromolecule,[7-10] is relatively higher in carbon and hydrogen and lower in oxygen content than are cellulose and hemicellulose, and it has the highest potential heat content of the three.[11] Hydrolysis of hemicellulose to mono- and oligosaccharides can be accomplished with either acids or enzymes under moderate conditions.[12-14] Unlike hemicellulose, cellulose is resistant to hydrolysis. Cellulose fibers generally consist of a highly ordered crystalline structure of cellulose surrounded by a lignin seal that becomes a physical barrier to easy hydrolysis. The secondary hydroxyl-linked polysaccharides are difficult to hydrolyze completely because of the inherently more resistant β-1.4-glucan materials. The easily hydrolysable

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portion of cellulose (amorphous region) is about 15% and the resistant residue (crystalline cellulose) about 85%. Crystalline cellulose may be hydrolysed by strong acid, but this also causes degradation of the glucose monomer. The crystalline structure and lignin barrier limits cellulose hydrolysis by either acids or enzymes.

A. Enzyme Degradation

Enzymes (cellulases) are specific catalysts that convert cellulose into glucose with little additional byproduct. However, cellulase has no effect on lignin and therefore the cellulose is not accessible to the enzyme. Ladisch et al.[15] have described an organic solvent pretreatment of cellulosic residues, followed by a cellulase hydrolysis process that yields 90 to 97% conversion of the residue to glucose.

The rate of enzymatic hydrolysis of cellulose is dependent upon several structural features of the cellulose. The cellulose features known to affect the rate of hydrolysis include (1) molecular structure of cellulose, (2) crystallinity of cellulose, (3) surface area of cellulose fiber, (4) degree of swelling of cellulose fiber, (5) degree of polymerization, and (6) associated lignin or other materials. Among these, the surface area and crystallinity of the cellulose are considered the most important for successful enzyme hydrolysis. The surface area is assumed important because direct physical contact between the enzyme molecules and the surface of cellulose is a prerequisite to hydrolysis. The crystallinity of cellulose is considered an important structural feature because cellulolytic enzymes readily degrade the more accessible amorphous region of cellulose, but do not easily act upon the crystalline region. As the crystallinity increases, cellulose usually becomes increasingly resistant to further hydrolysis. However, the recent research of Fan et al.[16,17] suggests that surface area is not a major limiting factor at the late stages of enzyme hydrolysis of cellulose as previously believed.

The two studies of Fan, Lee, and Beardmore[16,17] focused on the effects of major structural features of cellulose on efficiency and rate of enzymatic hydrolysis. The research involved the pretreatment of Solka Floc® and commercial microcrystalline cellulose with several physical and chemical methods in order to generate cellulosic samples with a wide range of crystallinity indices, and specific surface areas. The cellulase enzyme was obtained from culture filtrate of Solka Floc®~ cellulose fermentation by *Trichoderma reesi* QM9414. Results from these data showed that a highly linear relationship between crystalline cellulose and the rate of hydrolysis exists for different samples. However, the cellulose surface area and rate of hydrolysis were not clearly related. Although both degree of crystallinity and the specific surface area of the cellulose affect rate of hydrolysis, the data of Fan et al.[16,17] strongly indicate that enzyme rate depends mostly on crystallinity, not surface. This critical research suggests that in order to produce a practical commercial process for cellulose conversion, pretreatment research should aim toward degradation of the cellulose fine structure.

A selected recent example of the enhancement of the enzymatic hydrolysis of paper after pretreatment is presented in the research paper of Castanon and Wilke.[18] This work determined the effects of the surfactant Tween® 80 on enzymatic hydrolysis of newsprint. Crude enzyme solution from culture filtrate of *Trichoderma reesei* QM 9414 fermentation of 1% Solka Floc® was used. The culture filtrate-enzyme solution was treated with EDTA (16 mM) and sodium acetate (2 M; pH 4.95). Hydrolyses were carried out at 45°C using 0.1% Tween 80 and 5% Wiley-milled newspaper suspension in the enzyme solution. The results of these experiments[18] show that both higher cellulose conversions and enzyme recoveries were achieved. In the Tween 80-added hydrolysis, larger fractions of enzymes (as compared to controls) remained in solution throughout the paper hydrolysis. Consequently, the levels of sugar were also increased throughout the hydrolysis. It was postulated that the Tween 80 hindered the immobilization of the enzymes by reducing their strength of adsorption. These results, and the relatively low

cost of many surfactants, show an excellent future for reducing the cost of enzymatic hydrolysis of cellulosic materials.

Cunningham et al.[19] recently reported on the modification of wheat straw (WS) to enhance cellulose saccharification by enzymatic hydrolysis. The authors[19] demonstrated that treatments of WS which remove pentosans or lignin make the WS cellulose more readily available to saccharifying enzymes. In these studies,[19] chemical, physical, and thermal processes were applied to WS and the modified products were assayed by enzymatic hydrolysis. Conversion yields of glucose from cellulose by enzymatic hydrolysis were improved when lignin contents of the WS were reduced to 10% or less. Extraction of WS with sodium hydroxide (4% solution) removed lignin and pentosans, which resulted in substrates higher in cellulose (ash and pentosan free) contents. A two-step extraction of ground WS with sodium hydroxide and sodium hypochlorite provided substrates in which 82-90% of the cellulose could be converted to glucose by cellulase. A fourfold increase in cellulose conversion was noted for WS passing a 0.7 mm screen during hammer milling. When WS was subjected to thermal pulping or autohydrolysis its pentosan, lignin, mineral, and alcohol-benzene soluble contents of the pulp decreased as calculated on the basis of components in the original WS. For example, autohydrolysis at 170° C for 30 min decreased pentosan content and increased fourfold the conversion of cellulose to glucose.

Another example of a promising approach for enhancing cellulose accessibility and enzymatic hydrolysis by wet milling can be found in the recent report of Kelsey and Shafizadeh.[19a] The substrates used in these experiments were Whatman® CF-11 cellulose powder, newsprint, white pine hearth wood, and lignocellulose. A commercial preparation of cellulase from *Trichoderma viride* was the enzyme source. The various cellulosic materials were enzymatically hydrolyzed in a flask containing buffer solution, glass beads, sand, stainless-steel beads, or a combination of the three, and agitated via a shaker bath. For comparison, ball-milled substrates also were hydrolyzed; the control consisted of the same substrates without physical pretreatment. Results from these experiments[19a] indicate that wet milling enhances hydrolysis rate and thereby provide a more extensive saccharification. The effectiveness of the wet-milling process was dependent upon the lignified matrix of the cellulose microfibrils, the grinding elements, and the oscillation frequency of the shaker. Wet milling the cellulose for 48 hr with 3.5 mm glass beads and 200 oscillations per min yielded 1031 mg reducing sugar per g substrate (93% saccharification) as compared to 483 mg (44%) for the ball-milled substrate and 253 mg (23%) for the unmilled material. When the lignocellulose was wet milled with cellulase for 24 hr, 529 mg sugar per g substrate (93% saccharification) could be obtained. This was about three times greater than the ball-milled lignocellulose (169 mg; 30%) and 10 times greater than the unmilled (52 mg; 9%) substrate. Wet-milled wood particles (60 mesh) gave 143 mg sugar per g wood (about 38% saccharification) in 48 hr, whereas ball-milled sample yielded 79 mg (21%) and the unmilled substrate 38 mg (10%).

B. Chemical Degradation

Cellulose and lignocellulose have been transformed with alkali, acid, ethylamine, and ammonia.[20] The treatment of cellulose with sodium hydroxide solutions above 20% causes extensive swelling and separation of structural elements. Treatment of cellulose with liquid monoethylamine, followed by extraction with hexane or by evaporative procedures, yields a highly swollen product termed "decrystallized cellulose".[21,22] Many reviews on the alkali effect on wheat straw is referenced by Millett et al.[20] There is a wide range of differences in the manner in which alkali or ammonia affect the cellulose in wood chips, woodmeal, rice straw, and wheat straw, due primarily to the extent of lignification in the plant materials treated.[23-29]

C. Physical Methods

Lignin is one of the major deterrents to widespread utilization of lignocellulosic residues for microbial conversion. Of great economic importance is the degree of delignification needed to effect reasonable levels of carbohydrate utilization. Recent data encourage the use of chemical pretreatment of lignocellulosic material (in lieu of more expensive pulping) for disruption of the lignin-carbohydrate complex. The data show that complete delignification may not be required for maximum levels of carbohydrate utilization.[20-31] For example, Millett et al.[20] show the degree of delignification required to attain 60% in vitro carbohydrate digestibility from various woods: White birch, 25%; Red Oak, 35%; Red pine, 65%; and Douglas Fir, 73%.

1. Steaming

Other commonly used treatments to degrade lignocellulosic residues include steaming, grinding, irradiation, temperature, and pressure. Steaming has been used successfully in the production of a wood sugar molasses called Masonex. Masonex is obtained by concentration of the wash liquors from the manufacture of hardboard.[32]

2. Grinding

Grinding wood to a small particle size markedly enhances its susceptibility to chemical, microbial, and enzymatic influence. Vibratory ball milling of wood is one of the most effective means of generating small wood particles. Vibratory ball milling has been shown to increase cellulose digestibility of wood and forages by rumen bacteria.[33,34] Ball milling yields high concentrations of wood-sugar when the grinding is performed in the presence of hydrogen chloride gas or concentrated sulfuric acid. When grinding is followed by the addition of pulping chemicals, the products differ primarily in degree of delignification and perhaps the point of lignin removal or polymer swelling.

3. Irradiation

Gamma rays and high-velocity electron irradiation substantially improves the digestibility of wood or straw by microorganisms.[35,36] Electron irradiation increases both the rate of cellulose hydrolysis and subsequent sugar yields, under certain specific conditions.[37] Photodegradation also induces structural alteration within cellulosic materials.[38] Photodegradation involves exposure of polysaccharides to high-intensity ultraviolet light (3650Å) in the presence of sodium nitrite. Rogers et al.[39] reported up to a tenfold increase in the rate of biodegradation of a variety of cellulosic materials by using photodegradation pretreatment. The cost of irradiation makes its use in biomass conversion prohibitive. The estimated 1975 cost of irradiation was well over $100 per ton of material at dosages of 10^8 rad.[15] A successful effort to reduce irradiation cost has been reported by Han et al.[40] Han and co-workers combined chemical pretreatment with low dosages of irradiation to solubilize cellulose in sugarcane bagasse, newspaper, cotton linter, cotton cloths, saw dust, and alpha cellulose powder. In these experiments, the cellulosic materials were treated with swelling agents and exposed to gamma radiation from cobalt 60 or cesium 137. Solubilization of cellulose depended on irradiation dosage, and the rate was enhanced by alkali. By combination of chemical pretreatment and irradiation, the irradiation dosage needed to solubilize cellulose were reduced to 50 to 300 Mrads. This dose range is nearly tenfold less than previously reported.

4. Thermal

Dry heat modifies cellulose structure for modest benefits. About 200° C is the optimum temperature to produce a maximal rate of acid hydrolysis. However, a 32-hr treatment is necessary to effect maximum hydrolysis of 35%, with a yield of 27% sugar.[41]

Freezing cellulosic materials at −75° C with repeated freeze-thaw cycles has been

reported to reduce both strength and degree of carbohydrate polymerization.[20] The energy required makes low temperature an unlikely commercial procedure.

5. Pressure

Treating plant materials with pressure causes substantial changes to cellulosic materials. The review of Millett et al.[20] tells how Sharkov and Levanova compressed a cotton hydrocellulose for 30 min at room temperature, and thereby doubled the quantity of material subsequently dissolved during an ethanolysis process. The solubility of spruce sulfite pulp sheets was increased from 12% to 54% by repeated compression between calendar rolls. Millett et al.[20] reported that Odinstsov and Beinart obtained substantial improvement in cellulose saccharification by pressure milling with 75% sulfuric acid.

D. Degradation by Microorganisms

Microbial degradation of lignin, cellulose, and hemicellulose from wood has been reviewed by Meier,[42] Liese,[43] and Wilcox.[44] Kirk[45] has comprehensively reviewed microorganisms that effect lignin degradation. The more recent reports of Crawford et al.[46,47] and Kirk et al.[48] discuss microbes and the degradation of ^{14}C-labeled lignins and lignocelluloses to $^{14}CO_2$ by fungi and actinomycetes. According to Ishikawa et al.,[49] phenol oxidase-rich white-rot fungi degrade more lignin than phenol oxidase-poor white-rot fungi. In studies on white-rot fungi that preferentially degrade lignin, Arden and Eriksson[50] divided the fungi into two groups based on their phenol oxidase reactions on kraft lignin agar plates. Group 1 fungi *Sporotrichum pulverulentum, Phanerechaete* sp. L-1, and *Polyporus dichrous* produced a low level of phenol oxidase.

Group 2 fungi *Meruluis tremellosus, Phlebia radiata, Pycnoporus cinnabarinus,* and *Pleurotus ostreatus* produced a high level of phenol oxidase. The results suggested that preferential lignin degraders are easier to find among group 2 than among those in group 1.

A procedure for extracting hemicellulose from ryegrass straw for the production of glucose isomerase and for using extracted straw residue for animal feed has been developed by Chen and Anderson.[51] The hemicellulose fraction of ryegrass straw was extracted with NaOH and used as a substrate for production of glucose isomerase by *Streptomyces flavogriseus.* About 15% of the total available hemicellulose is obtained by treating ryegrass straw with 4% NaOH for either 3 hr at 90°C or 24 hr at room temperature. *S. flavogriseus* grown at 30°C for 2 days on 2% straw hemicellulose produced intracellular glucose isomerase at a level of 3.04 units/mℓ of culture. The alkali-treated residue (after separation of hemicellulose) had approximately 75% higher digestibility and 20% higher feed efficiency for weanling meadow voles than did untreated straw.

Various cellulase productions on bagasse and rice straw, wheat straw, woody waste, and other plant materials by the fungi *Trichoderma reesei, T. viride,* and *Aspergillus niger* have been cited.[52-54] *T. viride* may be the most active enzyme producer. Toyama and Ogawa[54] used *T. viride* to produce several enzymes, including "cellulase onozuka," xylanase, cellulase, β-1,3 glucanase, chitinase, plus several others at lessers potency levels. Cellulase onozuka or *T. viride* grown on wheat bran or rice straw at various substrate concentrations yielded a 5 to 10% sugar solution after incubation at pH 5.0, 45°C for 48 hr. Mitra and Wilke[55] have described the production of *T. viride* QM9414 enzyme in a multistage continuous fermentation system. Another cellulase preparation, *Meicelase,* is being produced by cultures of *T. viride* on a solid substrate of wheat bran at Yodogawa factory of Meij Seika, Osaka, Japan. Recent studies demonstrated the utilization of cellulose, Solka Floc®, Avicel, and cellobiose, and the subsequent production of cellulases by *Thermomonospora* sp.[56,57]

Continuous heterogeneous catalysis by immobilization techniques are the most novel

approaches to fermentation processes seen in the past decade. Such technology is being used for antibiotics, enzyme, and acid production, denitrification and removal of heavy metals from waste water, electrode BOD sensors, alcohol and corn sweeteners production, and many others. There is a surge of research activity in this exciting and rapidly growing field. A recent example of immobilization technology for enzymatic hydrolysis of cellulose has been presented by Sundstrom et al.[58] In their work, a practical immobilized-microbial β-glucosidase with high activity and long half-life was develoed and employed to hydrolyze cellulose. In typical cellulase systems, cellobiose is an intermediate product from cellulose, and subsequently, the cellobiose is converted to glucose by β-glucosidase. Usually, these enzyme systems contain insufficient β-glucosidase to prevent accumulation of inhibitory cellobiose. Sundstrom et al. immobilized β-glucosidase from *Aspergillus phoenicis* by sorption on controlled-pore alumina with about 90% activity retention. The product lost only 10% of its original activity during an on-stream reaction period of 500 hr with cellobiose as substrate. The immobilized β-glucosidase was used together with *T. reesei* cellulase to hydrolyze cellulosic materials, such as Solka Floc~, corn stover, and exploded wood. Significant increase in glucose yield and greater conversions of cellobiose to glucose occurred when the reaction systems contained supplemental immobilized β-glucosidase.

III. FERMENTATION CHEMICALS FROM BIOMASS

The chemicals industry currently is based primarily on nonrenewable resources; however, the economic potential for fermentation production of chemicals increases as the availability of petroleum resources declines and their costs rise. To take advantage of a trend toward fermentation chemicals in market substitutions, ways must be developed to resuscitate the microbiological industry. New sophisticated biotechnological improvements in the fermentation industry are necessary to reduce the cost of chemical production.

The total production (in the U.S.) of butadiene, acetic acid, acetone, isopropanol, ethanol, butanol, methyl ethyl ketone, glycerol, maleic anhydride, and fumaric acid is approximately 6.5 million tons, with a market value of about 3 billion dollars.[59] Less than 5% of these chemicals is produced by fermentation. The fermentation processes already developed are based on easily fermentable substrates, such as sugar and starch; these substrates may account for as much as 70% of the total cost of production. To be commercially competitive, current methods of fermentation must improve the rates of substrate utilization, increase yield of products, increase the energy efficiency of recovery processes (distillation), and develop new sugar feedstock from forage crops and wood biomass.

A. Acetone and Butanol
1. Fermentation of Starches

Commercial development of acetone/butanol fermentation began in the early 1900s, and depended on the bacterial conversion of potato starch to the solvents. Corn starch was used as a raw material for acetone/butanol production after the discovery of *Clostridium acetobutylicum* by Weizmann.[59] The genus *Clostridium* consists of spore-forming rod-shaped anaerobic bacterium isolated from soil, manure, roots of leguminous plants, cereals, decayed wood, corn stalks, sewage, or river-bottom mud. *C. acetobutylicum* has been used to produce acetone and butanol from various carbohydrate sources, including various grains, nuts, sugars, and food wastes. The most commonly used carbohydrate feedstock for *C. acetobutylicum* fermentation is corn starch.[60-63]
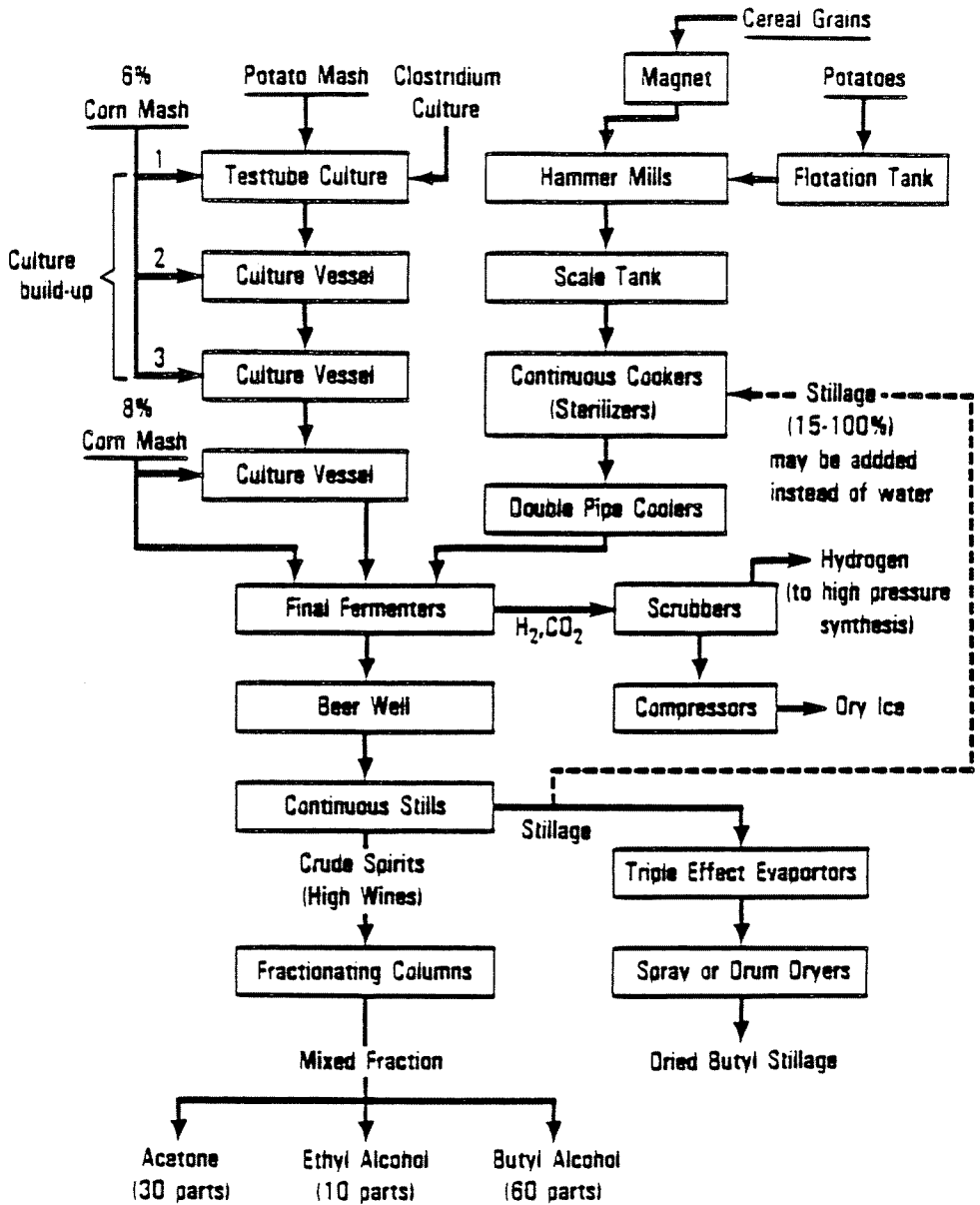
FIGURE 1.   Fermentation of starch to acetone and butanol, flow sheet. (Data from Reference 59.)

A process flow diagram for the fermentation of corn starch to acetone and butanol can be seen in Figure 1.[59] For this process, cultures of *C. acetobutylicum* are grown on a starch-nitrogen medium, and the culture is built up to the desired number of cells. Corn is degermed and the kernels are ground to a coarse corn meal. The ground corn meal is mixed with water to a 6 to 10% w/ v consistency and cooked for 2 hr to sterilize the mash and solubilize the starch. After being cooled to 37° C, the corn mash is pumped into the final fermentation stage. The diluted corn mash is inoculated with *C. acetobutylicum* and incubated at 37° C under anaerobic conditions. After 48 to 72 hr of fermentation, the

Table 1
FEED VALUES OF WHOLE DRY CORN STILLAGE FROM THE ACETONE/BUTANOL FERMENTATION[59]

Percent dry matter in stillage	Yield (kg/100 kg dry grain)		Riboflavin
	Dry feed	Protein	(μg/g)
2.32[a]	36.8	10.8	41
0.34[b]	8.6	1.0	6
1.95[c]	31.0	9.20	46
0.31[d]	4.9	1.36	16
1.68[e]	26.6	7.85	31

[a] Solids after evaporation of whole stillage.
[b] Dried solids on 40-mesh screen.
[c] Solids through 40-mesh screen.
[d] Solids after centrifugation of material through 40-mesh screen.
[e] Dried effluent from centrifuge.

beer, containing about 2.5% mixed solvents, is pumped to a distillation column and the solvents are recovered by further fractionation. The bottoms are concentrated by evaporation and dried for stillage recovery. Butanol, acetone, ethanol, carbon dioxide, hydrogen, and riboflavin-containing feeds are the major products from the starch fermentation. Solvent ratios of butanol (6), acetone (3), and ethanol (1) are obtainable. Yields are 1 kg of mixed solvents from 4.3 kg of corn or 1 kg of mixed solvents from 2.9 kg of starch. The solvent ratio and yield depends on the feedstock, the bacterial culture, the contaminants, the recycle of stillage, and the added chemicals. The stillage can be used as animal feed.[59] Feed values of whole dry stillage from the acetone/butanol fermentation of whole ground corn is shown in Table 1.[59]

2. Fermentation of Sugars

Several strains of Clostridia and Bacilli have been used to ferment sugar or molasses to acetone and butanol.[64-66] The most common sugar sources for the fermentation are invert and blackstrap molasses. Invert, or "high test", molasses is evaporated sugarcane juice that consists of glucose, fructose, and minor constituents. Blackstrap molasses is the syrup left after recovery of crystalline sucrose from concentrated sugarcane juice. Saccharolytic strains of *Clostridium* and *Bacillus* used in the acetone/butanol fermentation and their substrates are shown in Table 2.[59] Nitrogen for the fermentation process is supplied as ammonia or degraded protein (Table 2).[59] Inexpensive sources of degraded protein are cornsteep liquor, yeast autolysate, or de-oiled soybeans. Yields of 33 to 35% total solvents are obtainable in this fermentation, and the final solvent distribution is 65 to 68% n-butanol, 30 to 33% acetone, and 2 to 5% ethanol. *Clostridium acmylosaccharo-butylpropylicum, C. saccarobutyl-isopropyl-acetonicum, C. propyl butylicum*, and *C. viscifaciens* also produce significant (14 to 32%) amounts of isopropanol from invert molasses.

A process flow diagram of a typical acetone/butanol fermentation of sugars is similar to that seen in Figure 1. Heat-shocked cultures of *Clostridium* spores are incubated in a sterilized potato glucose medium at 31°C for 20 to 24 hr. The culture is then aseptically transferred to 600 ml of sterilized molasses mash and incubated for 20 to 24 hr. Next, the culture is transferred to molasses mash in a 4000-mℓ Erlenmeyer flask to ferment for an additional 20 to 24 hr. The 4000-mℓ ferment then is placed into an 18.9 kℓ (5000 gal) tank

Table 2
BACTERIA USED IN THE ACETONE/BUTANOL FERMENTATION[59]

Bacteria	Sugar source	Nitrogen added	Other addition
Bacillus butacone	Blackstrap molasses	Animal and vegetable protein	—
	Beet molasses	Complex nitrogen	—
B. saccharobutylicum	Invert molasses	—	$CaCO_3$
B. tetryl	Invert molasses	NH_3	—
Clostridium acmylosaccharo-butylpropylicum	Invert molasses	$(NH_4)_2SO_4$	$CaCO_3$, P_2O_5
Cl. celerifactor	Invert molasses	NH_3	$CaCO_3$
Cl. granulobacter-acetobutylicum	Molasses	Ammonium salts	Corn gluten, $CaCO_3$
Cl. madisonii	Cuban blackstrap molasses	NH_4OH, $(NH_4)_2SO_4$	$CaCO_3$
	Blackstrap molasses	NH_3	—
Cl. propyl butylicum	Invert molasses	NH_3	$CaCO_3$
		$(NH_4)_2SO_4$	$CaCO_3$, K_2HPO_4, $MgSO_4$
Cl. saccharoacetobutylicum	Cane molasses	Degraded protein	—
	Louisiana molasses	$(NH_4)_2SO_4$	$CaCO_3$
	Cuban molasses	$(NH_4)_2SO_4$	Gluten meal
	Invert molasses	NH_3	—
	Blackstrap molasses	NH_3	—
	Molasses	NH_3	—
Cl. saccharobutylacetonicum	Blackstrap molasses	$(NH_4)_2SO_4$	Corn gluten
Cl. saccharobutylacetonicum-liquefaciens	Blackstrap molasses	$(NH_4)_2SO_4$	$CaCO_3$, P_2O_5
	Cuban molasses	$(NH_4)_2SO_4$	$CaCO_3$, P_2O_5
	Molasses	NH_3	—
Cl. saccharoacetyoper-butylicum	Invert molasses	$(NH_4)_2SO_4$ NH_4OH	$CaCO_3$, P_2O_5 P_2O_5 •
Cl. saccharobutylicum	Blackstrap molasses	—	$CaCO_3$
Cl. invertoacetobutylicum	Louisiana molasses	Ammonium salts	Alkalies
Cl. saccharobutyl-isopropyl-acetonicum	Invert molasses	Degraded protein	—
Cl. viscifaciens	Invert molasses	—	$CaCO_3$

and incubated for future addition into the final fermentation tank. The molasses to be used in the final fermentation is mixed with water and steam to a 5 to 7% sugar concentration. The mash is sterilized at 107°C for 60 min in continuous cookers. After cooling to about 32°C, the mash is pumped into a final fermentor of 2.3 to 19 × 10⁵ℓ (60,000 to 500,000 gal) capacity and incubated with the prepared culture. The fermentation proceeds at 31°C under anaerobic conditions for 36 to 48 hr. At this time, the fermented beer (which contains about 2% mixed solvents), is pumped to a distillation column for solvents removal and concentration. A 40% solvent mixture is taken off overhead and distillers' slops are removed as bottoms. The solvents are further concentrated by fractional distillation. Stillage obtained from the initial distillation step is concentrated by evaporation and dried.

The major products formed in the acetone/butanol fermentation of sugars are the same as those in the starch fermentation. The solvent ratio yield depends on the same factors noted above for the starch fermentation. In the normal fermentation of sugars, a ratio of 34 parts butanol, 15 parts acetone, and 1 part ethanol can be obtained. The expected yield is 15.6 kg of mixed solvents from 100 kg of molasses. The stillage, from such a fermentation, is suitable for admixing with other materials for use as animal feed.

Table 3
FEED VALUES OF MOLASSES STILLAGE FROM THE ACETONE/BUTANOL FERMENTATION[59]

Type of molasses	Percent dry matter in stillage	Yield (kg/100 kg molasses)		Riboflavin (μg/g)
		Dry feed	Protein	
Invert[a]	1.15	17.7	6.5	52
Blackstrap[a]	2.71	28.6	6.0	38
Invert[b]	0.17	2.6	2.0	49
Blackstrap[b]	0.22	2.3	1.3	27
Invert[c]	0.96	14.8	4.1	54
Blackstrap[c]	2.50	26.3	4.2	37

[a] Total solids after evaporation of stillage.
[b] Solids recoverable by centrifugation.
[c] Dried effluent from centrifuge.

Recoverable feed values of stillage from acetone/butanol fermentation of sugars is seen in Table 3.[59] Also, the stillage may be recycled to the fermentation process to supply additional nitrogen and buffer substances. The stillage may be further concentrated, dried, and burned to an ash high in potassium, or used as a binder in foundry work.

Significant quantities of acetone and butanol are being produced in such countries as South Africa where fermentable biomass is relatively inexpensive.[67] Worldwide interest is now developing in the area of cellulosic waste conversion to butanol and other oil-sparing solvents and chemicals. Recent studies[68] on biological production of organic solvents from cellulosics involve conversion of animal feedlot residues. The process plan involves an alkali pretreatment of cattle feedlot residues followed by addition of a high-temperature actinomycete *Thermoactinomyces* sp., for cellulase production. The third and final step involves cellulase hydrolysis of the bulk residue, with subsequent fermentation of the sugar syrup by *C. acetobutylicum*. Preliminary economic evaluation indicates that, with present knowledge, butanol can be produced for about 35¢/lb.

Wang and coworkers have described significant new research data based upon the *C. acetobutylicum* fermentation.[69,70] Their experiments involve the use of corn meal medium with various *C. acetobutylicum* strains; these strains are capable of producing mixed solvents near theoretical maximum yields, i.e., 1.05 and 2.26 g/ℓ for acetone and N-butanol, respectively.

B. Acetic Acid

Acetic acid can be produced by biomass fermentation in five different well-known methods.[59] (1) anaerobic gasification of biopolymers to methane and CO_2, followed by methanolic carbonylation to acetic acid. This method essentially involves an anaerobic digestion to produce methane followed by introduction of the methane gas into a standard methanol carbonylation facility. (2) Anaerobic yeast fermentation of hydrolyzed biopolymers to ethanol, followed by oxidation to acetaldehyde and then to acetic acid. This second fermentation method of producing acetic acid involves well-known technology and may be the most favorable. First, ethanol is produced via a standard fermentation and is subsequently oxidized to produce acetaldehyde. To produce acetic acid from acetaldehyde, oxygen-enriched air and acetaldehyde are fed into a reactor at 66°C and 101.3 kPa, where they undergo a three-step chain reaction. The process is about 95% efficient with very few byproducts. (3) Anaerobic yeast fermentation of hydrolyzed biopolymers to ethanol, followed by aerobic bacterial

fermentation to acetic acid. The third method is the process currently used for vinegar production.[71] A conventional acetic acid process can be seen in Figure 2.[59] In this process, molasses, nutrients, and 1% ethanol are used to start a submerged aerobic fermentation. The concentration of ethanol is kept at about 1% until the acetic acid concentration nears 10 to 11%. This mixture of extractant and acetic acid is then put through a distillation chain to recover both. A major drawback to this process for acetic acid production is the energy-intensive distillation step, which adds substantially to the cost of acetic acid production. (4) Anaerobic bacterial homofermentation of biopolymers is the fourth method that has generated much interest in recent years. However, to date, no commercial technology using such microorganisms has been developed. Balch et al.[72] described a new genus of fastidiously anaerobic bacteria that produce a homoacetic acid fermentation, not from biopolymers. The type species, *Acetobacterium woodii*, ferments fructose, glucose, lactose, glycerate, and formate. In these fermentations, hydrogen is oxidized and CO_2 is reduced to acetic acid. Schoberth[73] has demonstrated the formation of acetic acid by cell-extracts of *Acetobacterium woodii*. (5) The fifth method consists of anaerobic bacterial heterofermentation of biopolymers with simultaneous production of ethanol and other acids. Heterofermentation of carbohydrates to acetic acids presents several purification problems. The separation and purification problems exist in any of the five fermentation processes mentioned; however, these problems are multiplied in heterofermentation by the presence of other organic products and a concomitant lower yield of acetic acid.

Increasing research is being conducted on the production of acetic acid by celluloytic anaerobes and particularly mixed culture fermentation. Wang et al.[69] reported studies on ethanol and acetic acid production by the cellulolytic anaerobe, *Clostridium thermocellum*, in cellulose-packed bed fermentors. The bacterium cellulose degradation was 67%, with a yield of 2.4 g/ℓ acetic acid from 110 g/ℓ of cellulose. Brooks et al.[74] conducted a mixed culture fermentation of cellulose (microcrystalline) at 55°C that yielded acetic acid as the major product, plus ethanol, 2,3-butanediol, and CO_2. One very interesting mixed culture fermentation that may ultimately determine the flow of cellulose carbon to rumen fermentation products involves two rumen bacteria, *Ruminococcus flavefaciens* and *Methanobacterium ruminantium*. The anaerobic cellulolytic rumen bacterium. *R. flavefaciens* normally produces succinic acid as a major fermentation product with acetic and formic acids, H_2 and CO_2. However, when the two rumen bacteria mentioned above are grown together on cellulose, acetic acid is the major fermentation product.

C. Citric Acid

Commercially, about 90% of citric acid is produced by either submerged or surface fermentation. Most new fermentation plants use submerged fermentation. About 400 million lb/year (180 million kg/year) of citric acid are produced in the U.S. and an additional 150 million lb/yr (68 million kg/year) in the rest of the world. In surface fermentation, spores of the fungus *Aspergillus niger* are inoculated on the surface of an appropriate medium in large pans that are about 2 to 3 in. deep.[75] After inoculation, the shallow pans are incubated still at 25 to 30°C for a total of 7 to 10 days; at this time, the fermentation is complete. The yields of citric acid based on the amount of sugar consumed depend on the substrate.[75-78] Table 4 shows examples of yields of citric acid from submerged and surface fermentation of various sugars by *A. niger*.[59] These yields are for the fermentation step only and range from about 70% in 4 days to 95% in 6 to 14 days. After the surface culture fermentation is complete, the liquor is decanted, the mycelial mat is washed, and the wash liquid is added to the fermentation solution. The only major difference between submerged and surface fermentations is that air must be supplied to the submerged culture at a rate of 0.5 to 1.5 VVm. The pH from both types of
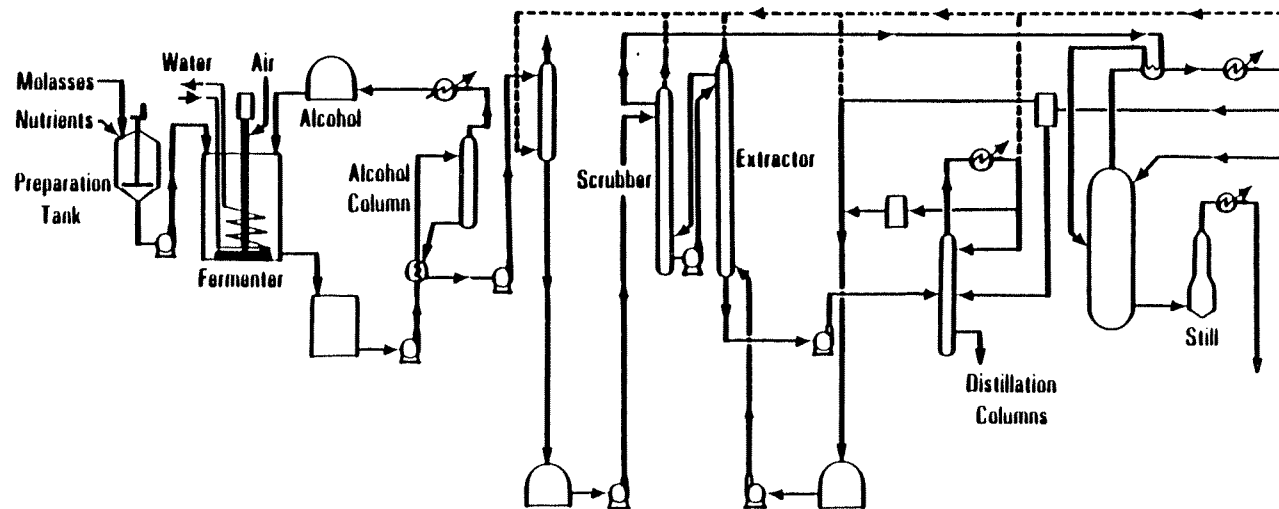
FIGURE 2. Conventional molasses fermentation to acetic acid, flow sheet. (Data from Reference 59.)

Table 4
LABORATORY PRODUCTION OF CITRIC ACID
BY *ASPERGILLUS NIGER*[59]

	Submerged fermentation		Surface fermentation	
Substrate	Yield (%)	Time (hr)	Yield (%)	Time (hr)
Sucrose	80	165	63	216
Glucose	71	168	90	200
Cane molasses	64	192	70	240
Beet molasses	64	216	50	240

fermentation drops from 4 to 2 or below during a 5 to 14 day fermentation. The media are the same for each type of fermentation. The sugar to be fermented is present in concentrations of 15 ro 25% w/v. Nitrogen is incorporated into the medium as an inorganic salt, or it is supplied in the substrate (molasses) in an organic form with concentrations no higher than 0.08 to 0.09% w/v. Phosphate, iron, manganese, magnesium, and zinc must be carefully controlled in the medium to assure good citric acid yields. Magnesium is usually supplied as $MgSO_4 \cdot 7H_2O$ at about 0.1% and potassium as KH_2PO_4 at 0.05 to 0.2%. Iron concentrations should not exceed 2 mg to 5 mg/ℓ of medium.

Other fermentative methods and microorganisms have been reported to be efficient in citric acid production. For example, Usami and Fukutomi[79] produced 50 to 60% citric acid yield/sugar equivalent in 3 days on solid media of sugarcane or pineapple molasses using *A. niger*. Hang et al.[80] demonstrated the production of 3.5 to 12.3 g/ℓ citric acid from a brewery waste (spent grain liquor), using the fungus *Aspergillus foetidus*. The Takeda process uses a *Candida* yeast growing on paraffins in long, deep tanks (60-metric-ton capacity). The citric acid is continuously precipitated as the calcium salt. The Takeda fermentation is the only alternate method used commercially today. However, noncommercial methods using other *Candida* species, *Candida oleophila*, and *Bacillus licheniformis* have been shown to produce citric acid from fructose or glucose.[81-83] Citric acid is recovered from the fermentation and purified by one of three commonly used methods: (1) direct crystallization after concentration of the filtered liquor, (2) precipitation as calcium tetrahydrate, or (3) solvent extraction. Precipitation is the most commonly used method for citric acid recovery from fermentation. Figure 3 shows a schematic diagram for the production of citric acid via molasses fermentation.[59] The fungal molasses fermentation converts 35–65% (w/w) of the sucrose or glucose to citric acid.

D. 2,3-Butanediol

The large-scale commercial fermentation of 2,3-butanediol (also called 2,3-dihydroxybutane) has never been fully accomplished. Fulmer et al. first demonstrated the commercial feasibility of producing the compound via fermentation by *Aerobacter aerogenes*.[84] Major research efforts to produce synthetic rubber during World War II brought about renewed interest in butadiene production. During this period, several universities, the Northern Regional Research Center, U.S.D.A., the National Research Council of Canada, and several commercial firms coordinated their search for an economical means of producing butadiene. The work focused on the *Aerobacter* and *Bacillus polymyxa* fermentations to produce 2,3-butanediol, and its subsequent conversion to butadiene. In the 2,3-butanediol fermentation, glucose is broken down to pyruvic acid, which is further metabolized to 2,3-butanediol.
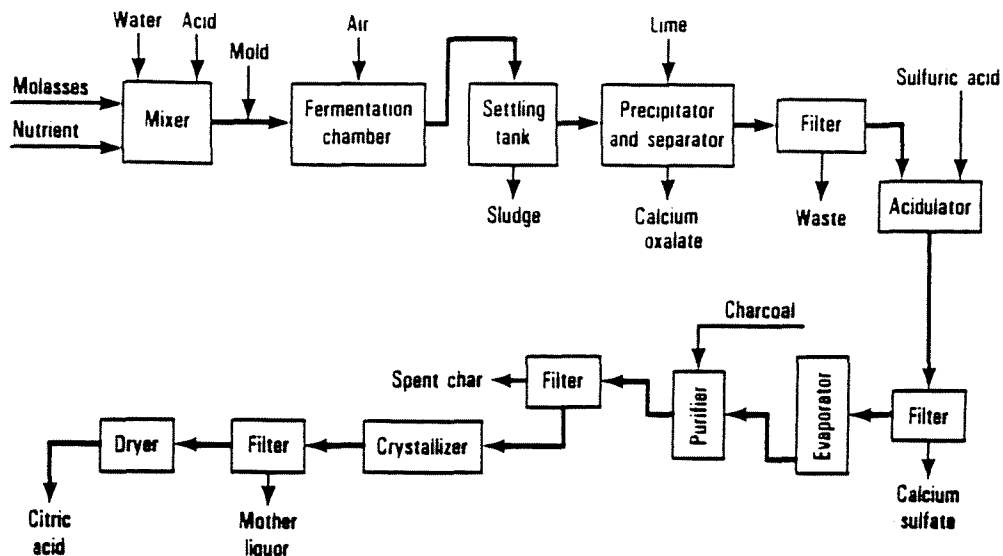
FIGURE 3.   Molasses fermentation to citric acid, flow sheet. (Data from Reference 59.)

Microorganisms that produce 2,3-butanediol from various raw materials are shown in Table 5. Although bacteria produce the major amount of 2,3-butanediol via fermentation, yeasts are also effective.[85] *Bacillus subtilis, Aerobacter aerogenes,* and *Serratia marcescens* produce significant quantities of 2,3-butanediol from hydrolyzed starch, some 35 lb butanediol per 100 lb starch.[86] Early investigations by Perlman[87] involved the production of 2,3-butanediol from acid hydrolyzates of hard and soft woods. In these studies, *Aerobacter aerogenes* fermentation yielded from 24 to 30% 2,3-butanediol depending upon the type of wood utilized.

Extensive pilot-plant research on the potential commercial production of 2,3-butanediol via fermentation has been reported by Wheat et al.[88] Wheat[89] designed a commercial plant to produce 4600 kg (10,200 lb) of 2,3-butanediol per day using 27,000 kg (60,000 lb) of molasses. Wheat also reported on several large-scale, pilot-plant experiments using *A. aerogenes* and *Pseudomonas hydrophilia* to ferment sugar beet molasses mash. *A. aerogenes* fermented 95 to 99% of the sugar and *P. hydrophilia* from 94 to 97%. Fermentation efficiencies ranged from 77 to 97% with *A. aerogenes* and from 76 to 98% with *P. hydrophilia.*

E. Propionic Acid

Propionic acid is a major end-product of glucose fermentation by *Propionibacterium* species. Co-products are acetic acid and CO_2. The fermentation involves the reduction of two pyruvic acid molecules to propionic acid, with the oxidation of a third molecule to acetic acid and CO_2.

Significant research has been conducted on the bioconversion of propionic acid to acrylic acid by *C. propionicum* from renewable resources.[69,86] Acrylic acid is a high-volume industrial chemical in high demand (about 1 billion lb/yr). Two anaerobic organisms, *Peptostreptococcus elsdenii* and *C. propionicum,* accumulate this acid as an intermediate. With *C. propionicum,* lactate is converted to acrylate, then to propionate via activated CoA thio esters. Acrylate production is stimulated by sodium lactate.

High yields of propionic acids by fermentation of wood sugars have been obtained by the Columbia Cellulose Co., Ltd., of Canada. Their pilot-plant process utilizes sulfite

Table 5
BACTERIA USED TO PRODUCE 2,3-BUTANEDIOL[59]

Bacteria

Aerobacter aerogenes	Beet, cane, glucose: citrus molasses; blackstrap sucrose; acid-hydrolyzed cornstarch; wood hydrolysate; acid-hydrolyzed wheat; sulfite waste liquor
Bacillus polymyxa	Beet, citrus, cane molasses; cornstarch; barley sulfite waste liquor; whole wheat sulfite waste liquor
Bacillus subtilis	Blackstrap; cane and beet molasses, glucose
Pseudomonas hydrophilia	Blackstrap; cane and beet molasses; glucose; sulfite waste liquor
Serratia marcescens	Beet molasses; sulfite waste liquor; glucose
Aeromonas liquifaciens	Cane molasses; blackstrap

waste liquor fermented by *Propionibacterium arabinosum* ATCC 4968. The feed contains 25 g/ℓ of fermentable sugars. The fermentation is carried out at 35 to 38°C for 54 hr in limestone packed column fermentors. Primary products are propionic acid and acetic acid in the ratio of two to one, respectively.[90]

F. Glycerol-Succinic Acid

Oura[35] presents detailed information regarding the formation of glycerol and succinate by yeast. Interestingly, the formation of glycerol appears to supply neither energy nor building units for the yeast cells in the fermentation.[91] The glycerol fermentation is known as the Neuberg 2nd and 3rd forms, in which glycerol accumulates in the fermentation. Two oxidation steps are involved in glycerol formation from glucose, and the redox-balance is achieved by the formation of two units of glycerol. Apparently, there is a direct correlation between redox-balance of the yeast cell and the formation of glycerol. When yeasts metabolize glucose under aerobic conditions, no glycerol is formed. Under these circumstances, the respiratory chain is functioning and transfers electrons to O_2 with no excess of $NADH_2$. Excessive $NADH_2$ is oxidized in the formation of glycerol, yielding a balance in the redox state of the cell.

Two mechanisms have been proposed for the formation of succinate in yeast during anaerobic fermentations. One is succinate formation via the normal oxidative mechanism of the TCA cycle, and the other is via a reductive pathway with malate and fumarate as intermediates.[92,93] The formation of succinate is considerably lower during the anaerobic growth of the yeast as compared to the fermentation stage of the yeast metabolism. The level of reduced nucleotides during growth is low, whereas they increase strongly during the yeast fermentation stage. When the level of nucleotides is high, during the fermentation stage, pyruvate is metabolized to oxalacetate via an activated pyruvate carboxylase, and then the TCA cycle will function actively.[94] The TCA cycle intermediates now accumulate as succinate and are excreted into the medium. As the formation of succinate occurs, so does an excess of reduced respiratory nucleotides ($NADH_2$). This excessive $NADH_2$ is oxidized in the formation of glycerol, yielding a balance in the redox state of the cell.

G. Fumaric Acid

Fumaric acid is produced principally by the fermentation of glucose or molasses with species of the genus *Rhizopus*. Rhodes et al.[95] demonstrated fumaric acid yields of 60 to

70% in 3 to 8 days in shaken flasks containing 10 to 16% glucose or sucrose, or the partially inverted sucrose of molasses. Although fumaric can be produced in high yields by fermentation, it is produced commercially as a byproduct in the manufacture of phthalic and malic anhydrides or by isomerization of malic acid with heat and catalyst. Several chemicals can be produced from fumaric acid, including malic acid, and maleic anhydride.

H. Lactic Acid

Griffith and Compere[96] describe a fixed-film system for continuous lactic acid production from wastewaters of the pulp, paper, and fiberboard industries. These wastewaters contain readily recoverable sugar polymers. Their fixed-film unit (2 inches × 6 inches) was seeded with Lactobacilli and lactose fermenting yeasts (kefir culture). The wood molasses substrate (wastewater concentrate) was pretreated with cellulase, a diastase, and hemicellulases. With a continuous feed rate of 60 g/ℓ wood molasses over the seeded fixed-film unit, 31 to 32 g/ℓ lactic acid yields were obtained. The production of calcium lactate from molasses by *Lactobacillus delbrueckii* has also been reported by Tewari and Vyas.[97]

I. Malic Acid

Pichia membranaefaciens is capable of converting fumaric acid to L-malic acid. Takao and Ho-Ha[98] describe malic acid production via mixed culture techniques. When *Rhizopus arrhizus* was grown on glucose for 2 to 3 days and then *Proteus vulgaris* was introduced to the fermentation, malic acid yields of 80% were obtained. These processes have not been commercialized.

IV. FERMENTATION FUELS

A. Methanol

Voluminous literature exists on the microbial production of methanol, and Foo[99] recently reviewed the basic considerations in search of microorganisms with potential for commercial production of methanol. Methanol occurs in nature as a breakdown product during microbial decomposition of plant materials and as a metabolite of methane-utilizing bacteria during growth upon methane. In recent years, methanol has become a potentially important carbon source for the production of single cell protein (SCP), enzymes, and amino acids.[100] Methanol also is a potential fuel for internal combustion engines, since it possesses cleaner burning properties and produces less pollution than hydrocarbon fuels. Also, a large volume of methanol is used as a solvent and as an intermediate in other chemical manufacture.

Methanol can be produced by the destructive distillation of wood; however, most methanol is derived from reaction of carbon monoxide with hydrogen.[101] Very little is known about the methods by which microorganisms produce methanol during decomposition of organic materials; however, reviews are available that explore this puzzle through utilization studies.[102,103] Methanol inhibition and the energy as well as reducing power requirements of methane oxidation present major problems to the microorganisms. Only small amounts of methanol are excreted by the cells during fermentation in various culture conditions.[104,105]
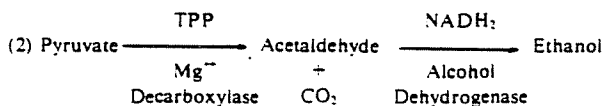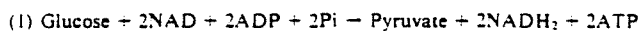
B. Ethanol

The uses of ethanol are many, including use as a solvent, in beverages, in food and feed via single cell protein (SCP), in hydrocarbon synthesis (via ethylene), as a gasoline dilutant (gasohol), and for biological energy (ATP). The basic steps of ethanol

production from grain, cellulose, or waste materials consist of (1) conversion of the grain starch or cellulose to fermentable sugar; (2) fermentation of the sugars to alcohol; and (3) separation of the resulting fermentation beer, which contains 6 to 12% ethanol, into substantially water-free ethanol. Sugars for ethanol production may be obtained from any feedstocks such as grains, watermelon and fruits, sugarbeets, sugarcane, sweet sorghum, and potatoes, or from cellulosic residues of corn, small-grain straws, wastepaper, sawdust, wood chips, forages (grasses), and cellulose-containing municipal waste.

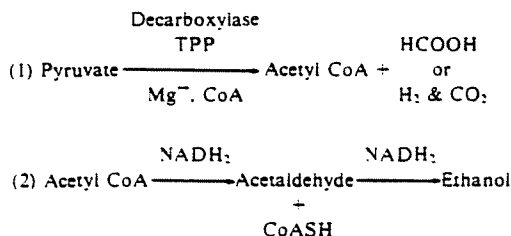### 1. Microbial Metabolic Routes for Ethanol Formation

The ability to produce ethanol from glucose is widely distributed among different yeast and bacteria. However, the yields of ethanol vary considerably, from about 2 mol of ethanol/mole of glucose fermented (characteristic of yeast) to considerably smaller ratios by several bacteria.[106] These variations are attributable to the operation of four different metabolic routes of ethanol formation, three of which involve pyruvic acid as an obligatory intermediate. Pyruvic acid may be produced from glucose by different metabolic sequences, such as Embden-Meyerhof glycolysis or Entner-Doudorff cleavage, with subsequent conversion to a C_2 chemical unit via decarboxylation to acetaldehyde or via a thioclastic reaction to acetyl coenzyme A. Reduction of either C_2 unit yields ethanol.

Type I. Glycolysis:

(1) Glucose + 2NAD + 2ADP + 2Pi → Pyruvate + 2NADH$_2$ + 2ATP

$$
\begin{array}{ccc}
& \text{TPP} & \text{NADH}_2 \\
\text{(2) Pyruvate} & \xrightarrow{\hspace{2cm}} \text{Acetaldehyde} & \xrightarrow{\hspace{2cm}} \text{Ethanol} \\
& \text{Mg}^{++} \qquad + & \text{Alcohol} \\
& \text{Decarboxylase} \quad \text{CO}_2 & \text{Dehydrogenase}
\end{array}
$$

The type I pathway of microbial metabolism of glucose via pyruvate and acetaldehyde leads to essentially quantitative conversion of glucose to ethanol and carbon dioxide. The yeasts are best known for utilizing this pathway, but bacteria are known that possess a yeast-like pathway and ferment glucose almost quantitatively to ethanol and CO_2.

Type II. Thioclastic reaction:

$$
\begin{array}{ccc}
& \text{Decarboxylase} & \\
& \text{TPP} & \text{HCOOH} \\
\text{(1) Pyruvate} & \xrightarrow{\hspace{2cm}} \text{Acetyl CoA} + & \text{or} \\
& \text{Mg}^{++}, \text{CoA} & \text{H}_2 \,\&\, \text{CO}_2
\end{array}
$$

$$
\begin{array}{ccc}
& \text{NADH}_2 & \text{NADH}_2 \\
\text{(2) Acetyl CoA} & \xrightarrow{\hspace{1.5cm}} \text{Acetaldehyde} & \xrightarrow{\hspace{1.5cm}} \text{Ethanol} \\
& + & \\
& \text{CoASH} &
\end{array}
$$

The Clostridia and Enterobacteriaceae cleave pyruvate to acetyl coenzyme A (CoA), with subsequent reduction to acetaldehyde and ethanol. For quantitative conversion of glucose to ethanol, H_2 production must be suppressed to provide the reducing power essential for ethanol production.

Type III. Entner-Doudoroff pathway:

```
              ATP                    NAD
    (1) Glucose─────── G-6-P ──────────── Gluconate-6-P ╮
                  Pyruvate ───────────── 2-oxo-3-        ╞ -H₂O
                      ÷          Deoxygluconate-6-P ╯
              Glyceraldehyde-3-P
    Pyruvate ───────────┘

    (2) Pyruvate─────────── Ethanol
```
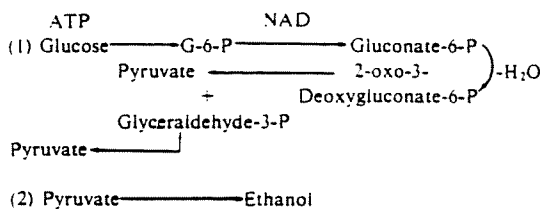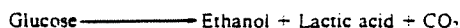
Zymomonas species give a similar fermentation balance to yeast, but ethanol derives from C-2, C-3, C-5, and C-6 of glucose with only half the energy yield.

Type IV. Heterolactic fermentation:

```
    Glucose ─────────── Ethanol ÷ Lactic acid ÷ CO₂
```

Heterolactive microorganisms are capable of glucose germentation to lactate and ethanol via xylulose 5-PO_4, which is subsequently cleaved to yield acetyl PO_4 and glyceraldehyde 3-PO_4. The latter is converted to pyruvate with subsequent reduction to lactic acid. The acetyl PO_4 is reduced to ethanol, utilizing the reducing power generated from the glucose to xylulose 5-PO_4 conversion.

2. Ethanol from Plant Biomass

Ethanol production from plant biomass is being studied extensively by various research laboratories throughout the world. Bellamy[107] and Brooks et al.[74] are pursuing the production of both SCP and alcohol from agricultural waste by utilizing various biological conversion processes. Their research also includes thermophilic bacteria that produce ethanol from xylose, mixed culture fermentation, and the use of *Clostridium thermocellum*. Wang et al.[69,70] are investigating the cellulolytic activity of mutants of *C. thermocellum* capable of unusual alcohol tolerance. These bacteria generate some 3 g/ℓ reducing sugars and 2 g/ℓ ethanol when they are grown on cellulose (10 g/ℓ) for about 75 hr. Various strains of *C. thermocellum* consume 8 to 66% of the cellulose in corn cob granules and therefrom produce reducing sugars from 1.38 to 2.95 mg/mℓ.

Bioconversion of wheat straw to ethanol was reported recently by Detroy et al.[108] These experiments involved chemical modification, enzymatic hydrolysis, and final glucose fermentation to ethanol by *Saccharomyces* sp. Native wheat straw (WS) was pretreated with various concentrations of H_2SO_4 or NaOH, followed by secondary treatments with ethylene diamine (EDA) and NH_4OH, and concluded with commercial cellulase saccharification. Conversion of the cellulosic component to sugar varied with the chemical modification step. Treatment solely with alkali yielded 51 to 75% conversions, depending on temperature. Acid treatment at elevated temperatures showed a substantial decrease in the hemicellulose, whereas EDA-treated WS (acid pretreated) had a 69 to 75% decrease in the lignin component. Acid-pretreated, EDA-treated straw yielded a 98% conversion rate. Alkali-NH_4OH-treated WS yielded an 83% conversion rate. Pretreatment of WS with 2% NaOH for 4 hr, coupled to enzymatic hydrolysis, yielded 76% conversion of the cellulosic component. The cellulase preparation yielded considerable quantities of xylose in addition to the glucose. Saccharified final WS materials were fermented directly with actively proliferating yeast cells.

Brazil has developed processes that utilize cassava roots, palm trees, sugarcane, and babassu coconut. The babassu coconut crop in Brazil alone could enable the production

of about 8 billion liters of ethanol annually. This coconut (23% starch) is produced at the rate of some 210 million tons a year, and theoretically it could be converted to nearly twice the 1980 ethanol production (4.3 billion liters) in Brazil.[109] Carioca and Scares[110] obtained a relative yield at 76% conversion rate of babassu flour (60% starch) to ethanol in their fermentation experiments. Their process involved gelatinizing the babassu starch at 80 to 85°C and then adding a heat-stable α-amylase. Complete saccharification was accomplished by glucoamylase treatment for 40 hr at room temperature. Yeast extract was added to the hydrolyzed coconut starch to support microbial growth. The fermentation was conducted at 28–30°C for 42 hr. After distillation, the yield was 90 mℓ of 92% purity ethanol from 250 g babassu flour in 1 ℓ H_2O.

The cassava plant has commanded considerable attention in Brazil as a starch resource for fermentation.[111] Cassava (also known as manioc or tapioca) contains 20 to 35% starch and 1 to 2% protein in its roots. The average crop production in Brazil is 13 tons of roots per hectare. The feasibility of alcohol from starch materials to compete with Brazil's already successful sugarcane process will depend principally upon the optimization of the liquefaction and saccharification steps of manufacture.
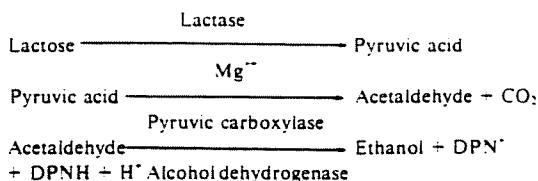
The problem of starch hydrolysis exists in the cassava plant, even though the cassava starch is readily susceptible to α-amylase. The cassava root fibers create a barrier to starch hydrolysis when whole roots are used for fermentation. The cassava root fibers can be removed via biological pretreatment with the cellulolytic microorganism *Trichoderma viride*. Menezes et al.[112] demonstrated that fermentation broth of a *Basidiomycete* and *T. viride* increased both the rate of sugar formation and degree of solubilization, with subsequent decrease in substrate viscosity.

3. Agricultural and Industrial Waste for Ethanol Production

Whey, a byproduct of cheese manufacture, has become a serious pollution problem. In 1974, about 32.5 billion pounds of whey were produced, half of which was disposed of as waste.[113] This residue represents 1.6 million lb of lactose, which can be utilized as a fermentation resource. O'Leary and coworkers[114,115] reported alcohol fermentations of a lactose hydrolyzed acid whey permeate (4.0 to 4.5% lactose) containing 30 to 35% solids. The fermentations of hydrolyzed whey were conducted for 13 days with *S. cerevisiae* and *Kluyveromyces fragilis* and resulted in maximal yields of 6.5 and 4.5% ethanol, respectively. The galactose generated during hydrolysis was not utilized. Roland and Alm[116] hydrolyzed whey permeate syrups, fortified them with nitrogen, and fermented the hydrolysates with *S. cerevisiae* var. *ellipsoideus*, resulting in a 12.5% v/v alcohol production. Fermentations were conducted with interval feedings of hydrolyzed whey permeate syrup; maximal alcohol yield occurred in 6 days. Galactose utilization by the yeasts was not measured; however, residual reducing sugars in the wines varied from 0.2 to 4.3%. Apparently, a wide variability exists between the fermentation capacity of *S. cerevisiae* strains to utilize galactose.

In 1938, Meyers and Weisberg[117] early research demonstrated the suitability of *K. fragilis* for manufacturing delactosed whey. Then ethanol was only a byproduct of the delactosed whey production and not considered to be of economic importance. At that time delactosed whey was used as an excellent quality animal feed supplement. Yeast fermented whey contains high concentrations of albumin, globulin, and minerals as well as vitamins contained in the yeast cells.[118] The most recent and well known commercial process for cheese whey fermentation to ethanol is the "Milbrew Process". This process was developed in 1972 by Milbrew Inc., Juneau, Wisconsin, partially under a research grant from the office of Research and Development, U.S. Environmental Protection Agency (EPA).[118] The Milbrew plant operated its yeast and ethanol production facilities in a continuous procedure in 1974–75 for the EPA. Milbrew Inc. has been quoted as being the only plant in the U.S. making commercial size quantities of yeast and ethanol

from whey.[118] The production of ethanol from cheese whey is accomplished mostly through yeast fermentation of lactose to pyruvic acid, acetaldehyde, ethanol, and carbon dioxide:

$$\text{Lactose} \xrightarrow[\text{Mg}^{++}]{\text{Lactase}} \text{Pyruvic acid}$$

$$\text{Pyruvic acid} \xrightarrow[\text{Pyruvic carboxylase}]{} \text{Acetaldehyde} + CO_2$$

$$\text{Acetaldehyde} \xrightarrow{\text{Alcohol dehydrogenase}} \text{Ethanol} + DPN^\cdot$$
$$+ \text{ DPNH} + H^\cdot$$

a. Direct Conversion-Mixed Cultures

The most thoroughly studied process for producing ethanol from agricultural waste is its enzymatic conversion to soluble sugars and subsequent fermentation to ethanol by yeast. Wilke et al.[119-121] and Cysewski and Wilke[122] have provided some preliminary economic evaluations on various principal cost elements involving enzymatic conversion techniques. The presentations discuss the distribution of costs associated with ethanol production (exclusive of raw material costs) from newsprint and wheat straw. The major cost is for saccharification, because the fermentor capacity required to produce sufficient quantities of fungal cellulase is 30 to 40 times that required to ferment the resulting sugars. Su and Paulavicius[123] have described the volumetric production efficiencies for alcohol production by fermentation of newsprint, wheat straw, and molasses. This efficiency in gram per liter-hour is significantly lower than the conventional molasses fermentation by yeast and is reflected in the conversion cost estimates.

In the usual fermentation process, cellulase is isolated and used to hydrolyze cellulose in a separate reactor. A more direct conversion, in which cellulase production, cellulose hydrolysis, and a fermentation are carried out simulataneously in a single operation, has been presented by Cooney et al.[124] In their scheme, the anaerobic, thermophilic bacterium, *Clostridium thermocellum*, is utilized to produce hexose sugar, ethanol, and acetic acid from corn residue, Solka Floc®, and cellobiose. *C. thermocellum* is unable to metabolize pentoses to ethanol; however, *C. thermosaccharolyticum* ZC can. Therefore, a combination of these two cultures can directly convert substantial cellulose and hemicellulose (hexoses and pentoses) to ethanol.[125,126] This mixed-culture system directly converts solka floc and ground corn stover to a mixture of ethanol and acetic and lactic acids.[125] Selected isolates of the two Clostridia species produce more ethanol and less acetic acid than the parents. These isolates grown in mixed culture yield 0.57 g total of ethanol, acetic, and lactic acids per gram of cellulose.[125] Mixed-culture systems of Clostridia also are being applied to the conversion of hardwoods to ethanol.[126] In this procedure, the cellulase-producing fungus *Thermoactinomyces* YX is combined with *C. thermocellum* for a single-step, high-temperature, saccharification fermentation.[127,128] Another direct conversion fermentation combines *Trichoderma* with another yeast.[129-132] The fungus-yeast fermentation was tested on municipal solid wastes, pulpmill primary sludge and digester rejects, and agricultural and forest residues. In such experiments, continuous removal of the glucose formed during hydrolysis substantially reduced product inhibition.

The production of alcohols from the hemicellulose in industrial wastes may be the least expensive way to produce liquid fuels by fermentation.[133] Hemicellulose-derived pentoses can be obtained easily in good yield from residues, using a relatively simple process.[134] Most yeasts (commonly used for alcohol production) lack kinases and D-xylose isomerase. Therefore, most yeasts cannot convert pentose sugars (e.g., D-xylose and L-arabinose) to ethanol; however, other microorganisms do. For example, xylan can be degraded by yeast of the genera *Aureobasidum*, *Cryptococcus*, and *Trichosporon*.[115]

and *Candida utilis* and *Candida albicans* utilize D-xylose.[131] *Clostridium thermo-saccharolyticum* ferments xylose to a mixture of ethanol, lactic acid, and acetic acids.[125] *Bacillus macerans* ferments xylose to a mixture of ethanol, acetic acid, and acetone.[136] *Aeromonas hydrophilia* produces ethanol and 2,3-butanediol from xylose;[117] both chemicals are suitable as fuel. *Fusarium oxysporum* and other *Fusarium* species degrade xylose and have been used in combination with the yeast *Saccharomyces cerevisiae*.[138-141] Several of these processes use a yeast to convert glucose to ethanol and later the fermentation is inoculated with *Fusarium* to produce additional alcohol from pentoses.

V. CONCLUSION

In general, the technologies are available for converting biomass into fuels and chemicals. However, significant improvements in existing and preceived technologies are required to rapidly achieve industrial acceptance. Cellulose is the biomass residue receiving the greatest attention now. Cellulose has been converted into glucose, protein, alcohol fuels, methane gas, solvents, and other chemicals. The major problem in the conversion of residues is their resistance to hydrolysis. Although hydrolysis of cellulose can be done, it is not yet done efficiently. Conversion of lignin to useful chemical products is a particularly challenging problem. Lignin stability appears to be one of the major hurdles to readily digesting cellulosic residues. Apparently, in animal feed, fuel production, and elsewhere, the degree of delignification of cellulosic residues determines success in obtaining desired products. Fungi, bacteria, algae, and yeast have been shown to degrade cellulosic residues to varying degrees. Current research involves the development of new and improved microorganisms capable of cellulosic digestion. Alcohol-tolerant yeast and bacteria, increased cellulase production by bacteria and fungi, and mixed-culture fermentation are among the approaches being researched. The most significant advances may come through new microorganisms developed through DNA transfer, recombinant DNA, cell fusion, and altered membrane compositions.

There are two questions, the answers to which shall put the subject of biomass conversion in the proper energy perspective: (1) what is the energy conversion efficiency? and (2) what is the time span needed before biomass conversion becomes the significant source of energy in the U.S.? Energy conversion efficiency, or net balance, is a technical parameter that invariably needs discussing when alternative energy resources are being evaluated. The amount of energy consumed depends on the raw materials that are being converted, variables in the conversion process and technology, and the desired end product. Liquefaction of coal has a 65% efficiency; the conversion of coal to high Btu gas, 60%; generation of electricity from coal, 35%; biomass fermentation to ethanol, 35%; and biomass gasification, 55%.[142] Nuclear generation of electricity has a 30% conversion efficiency. However, as important as net energy balance is, it is by no means the final determining factor as to whether an alternative resource is desirable. In Brazil, the government decided that biomass ethanol is to be used widely as a gasoline supplement and replacement, and so it is. Obviously, a process with comparatively low energy conversion efficiency may still be desirable because of a variety of other technical, economic, and sociopolitical considerations.

About 90% of our energy comes from oil, gas, and coal, with almost half from oil alone. These resources will continue to be the primary feedstocks for the chemical and fuel industry through the end of this century, and for as long thereafter as they remain the least expensive alternative. It is generally agreed that we cannot effect a major worldwide shift from direct or indirect use of fossil fuels until after the year 2000. Also, it should be remembered that this present time is not the first to experience energy transition in U.S. history. Until about 100 years ago, wood was our primary fuel resource. Then, in the post-Civil War years, wood and waterwheels gave way to coal, and within about a

generation or so, coal gave way to large-scale use of oil and natural gas. The important thing to note it that there has been a time span in excess of 50 years involved in the switch from one major energy source to another. Technology may speed up any future major energy shift, but basic changes have to occur in our energy production and utilization systems before there is much impact on the status quo.

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